Selective Cell Transplantation Using Bioabsorbable Artificial Polymers as Matrices

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● To date, selective cell transplantation has involved injecting cell suspensions into tissues or the vascular system. This study describes attaching cell preparations to biosrodable artificial polymers in cell culture and then implanting this polymer-cell scaffold into animals. Using standard techniques of cell harvest, single cells and clusters of fetal and adult rat and mouse hepatocytes, pancreatic islet cells, and small intestinal cells have been seeded onto biodegradable polymers of polyglactin 910, polyanhydrides, and polyorthoester. Sixty-five fetuses and 14 adult animals served as donors. One hundred fifteen polymer scaffolds were implanted into 70 recipient animals: 66 seeded with hepatocytes; 23 with intestinal cells and clusters; and 26 with pancreatic islet preparations. The cells remained viable in culture, and in the case of fetal intestine and fetal hepatocytes, appeared to proliferate while on the polymer. After four days in culture, the cell-polymer scaffolds were implanted into host animals, either in the omentum, the interscapular fat pad, or the mesentery. In three cases of fetal intestinal implantation coupled with partial hepatectomy, successful engraftment occurred in the omentum, one forming a visible 6.0 mm cyst. Three cases of hepatocyte implantation, one using adult cells and two using fetal cells, have also engrafted, showing viability of hepatocytes, mitotic figures, and vascularization of the cell mass. To date, no pancreatic islets have survived implantation. This method of cell transplantation, which we have termed "chimeric neomorphogenesis," is an alternative to current methods and requires further study.

INDEX WORDS: Transplantation; cell culture; fetal cell transplantation.

SELECTIVE cell transplantation of only those parenchymal elements necessary to replace lost function has been studied as an alternative to whole or partial organ transplantation.[1] In the past, cells have been harvested, dispersed into a suspension, and then inoculated into various tissues. We report attaching parenchymal cells from liver, intestine, and pancreas onto bioerodable artificial polymers and then implanting these polymer-cell scaffolds into animals as a novel method of cell transplantation.

MATERIALS AND METHODS

Polymers

Three synthetic absorbable polymers have been used to fabricate filaments and discs as matrices for cell attachment, growth, and implantation (Fig 1).

Polyglactin. This polymer was developed as absorbable synthetic suture material. It is a 90-10 copolymer of glycolide and lactide and is produced as Vicryl braided absorbable suture (Ethicon Co, Somerville, NJ).[2]

Polyorthoesters. The specific polymer used was 3,9-bis(ethylidene-2,4,8,10-tetraoxaspiro[5.5]) undecane copolymer with *trans*-1,4-cyclohexanedimethanol and 1,6-hexandiol in a molar ratio 2:1:1, respectively (SRI, CA).[3]

Polyanhydride. The specific polymer used was a polyanhydride of bis(1,4-carboxyphenoxy) propane and sebacic acid. It is biocompatible and has been used extensively in drug delivery applications.[3,4]

Polymer Configuration

Small wafer discs or filaments of polyanhydrides and polyorthoesters were fabricated using one of the following methods.

Solvent casting. A solution of the polymer (10% in methylene chloride) was cast on a branching pattern relief structure as a disc 10 mm in diameter for 10 minutes at 25°C using a Carver press. After solvent evaporation, a film 0.5 mm in thickness with an engraved branching pattern on its surface was obtained.

Compression molding. One hundred mg of the polymer was pressed (30,000 psi) into a branching pattern relief structure 10 mm in diameter. 0.5 mm discs were obtained.

Filament drawing. Filaments were drawn from the molten polymer (30 μm in diameter). Small flattened 1.0 cm tufts were used for the experiments.

Polyglactin 910. Multiple fibers of 90-10 copolymer of glycolide and lactide converging to a common base were fashioned from suture material of 0-Vicryl by fraying the braided end of the polymer (Fig 2). These branching fiber clusters were approximately 1.0 cm in height. The individual fibrils were 30 μm in diameter.

Animals

Young adult and fetal Sprague Dawley rats and C57 Bl/6 mice (Charles River Labs, Wilmington, MA) were used as cell donors for all experiments. The animals were housed individually, allowed access to food and water ad lib, and maintained at 12 hour light and dark intervals. Anesthesia was obtained with an intraperitoneal injection of pentobarbital (Abbott Labs, North Chicago, IL) at a dose of 0.05 mg/g and supplemented with methoxyflurane (Pitman-

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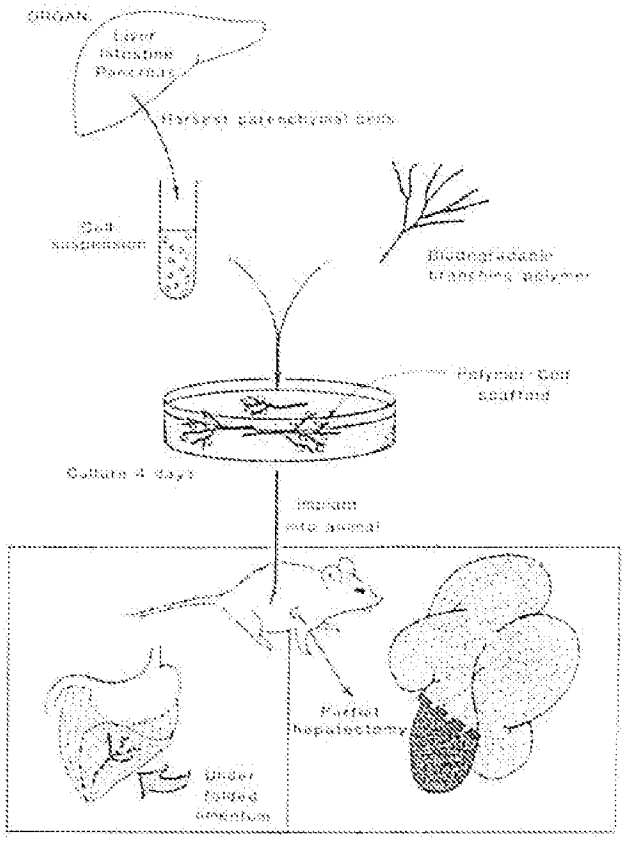

in the midline using sterile technique. The common bile duct was isolated, and the pancreas visualized. Two and a half milliliters of 2.0% type II collagenase was infused into the pancreas by injection into the common bile duct using the technique described by Gotoh et al.[5] After five minutes, the pancreas was transferred to a sterile hood for islet cell isolation. Briefly, the tissue was placed into a 25% Ficoll

the interscapular fat pad; (2) the omentum; and (3) the bowel mesentery (Fig 3).

Most animals underwent a partial hepatectomy to stimulate cell growth. Animals were sacrificed at day 3, 7, or 14 and the implants were examined histologically with hematoxylin and eosin. Polymers without cells served as controls. Polymer-cell scaffolds were exam-

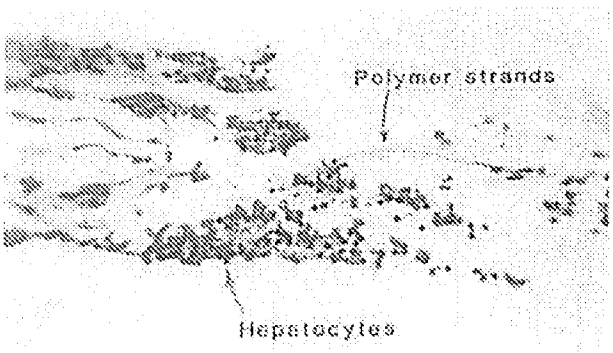

Fig 3. Hepatocytes attached to fibers of polyglactin 910 after four days in culture. Cells vary from healthy to degenerated and necrotic (Hematoxylin and eosin, original magnification ×172).

ined histologically after four days in culture and before implantation to assess cell attachment and viability.

RESULTS

Seventy-nine animals, including 14 adults and 65 fetuses, were used as donors for cell harvest; 115 polymer scaffolds were prepared for implantation. Sixty-six of these scaffolds were seeded with hepatocytes, 23 with intestinal cells and clusters, and 26 with pancreatic islets and cell preparations. Implantation was performed in 70 recipient animals. Fifty-eight were killed at seven days for histologic examination of the implant while three were examined at three days, and nine at 14 days after implantation.

Cell viability on the polymer scaffold at 3 to 4 days in culture varied with the type of polymer material used. Less than 10% of the cells were viable on the polyanhydride discs, whereas 80% of cells cultured on polyorthoester discs and filaments remained viable, and over 90% survived on polyglactin 910 (Fig 3).

Blood vessel ingrowth was noted three days after implantation with all of the polymer types and configurations. In the implanted fiber networks, new blood vessels formed in the interstices between the polymer filaments. The polymer discs showed capillary formation immediately adjacent to the polymer material. This angiogenic response accompanied an inflammatory infiltrate that displayed both an acute phase and a chronic foreign body reaction to the implanted polymers. The intensity of inflammation varied with the polymer type tested: polyanhydride elicited the most severe acute and chronic response although the inflammation surrounding branching fibers of either polyorthoester or polyglactin appeared proportionately greater than the disc configuration because of the greater surface area of exposed foreign material to host.

Histologic examination of liver cell implants in three animals showed evidence of successful engraftment of hepatocytes at seven days. Small clusters of healthy appearing hepatocytes were seen with bile canaliculi between adjacent cell membranes and some areas demonstrated mitotic figures. The cells were surrounded by an inflammatory response and blood vessels coursed around and through the cell clusters. Polymer material was seen immediately adjacent to the cells (Fig 4).

Successful engraftment of intestinal cells and clusters were observed in three animals. Histologic findings were similar to the hepatocyte implants with one exception (Figs 5A-C). On gross examination of the implant at seven days, a cystic structure approximately 6.0 mm in length was found at the implant site (Fig 5A) with polymer fibers splayed within its wall. Microscopic examination revealed well-differentiated intestinal epithelium lining the cavity with mucous and cellular debris within the lumen. One wall of the cyst contained polymer fibers, blood vessels, and inflammatory cells immediately adjacent to the intestinal epithelium (Fig 5B). The other wall included a muscular coating that suggested that the polymer held a small minced piece of fetal intestine as the origin of the cyst that eventually developed. The cyst displayed well-differentiated intestinal epithelium with mucous secreting cells (Fig 5C). Other clusters of intestinal epithelium demonstrated active mitoses.

No viable pancreatic islets or other cell types were found in any of the pancreas implants tested. However, all of the implants were accompanied by an inflammatory and angiogenic response similar to those noted with liver and intestine.

Control polymers implanted without prior cell seed-

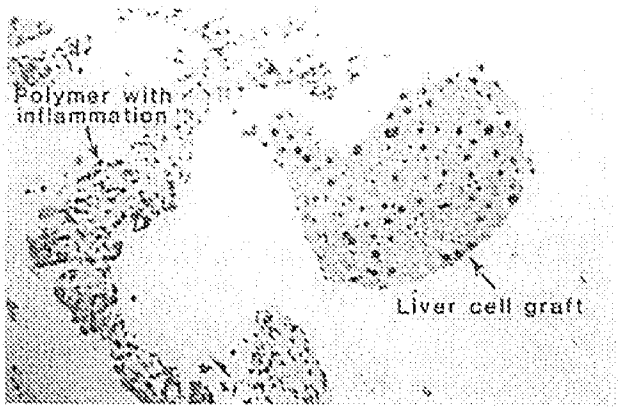

Fig 4. Implant of hepatocytes from adult rat donor into omentum. The polymer-cell implant has been in place for seven days before death. Hepatocytes are healthy and several mitotic figures can be seen. Blood vessels are present in the mass. To the left, an inflammatory infiltrate in the area of the polymer is observed (Hematoxylin and eosin, original magnification, ×172).

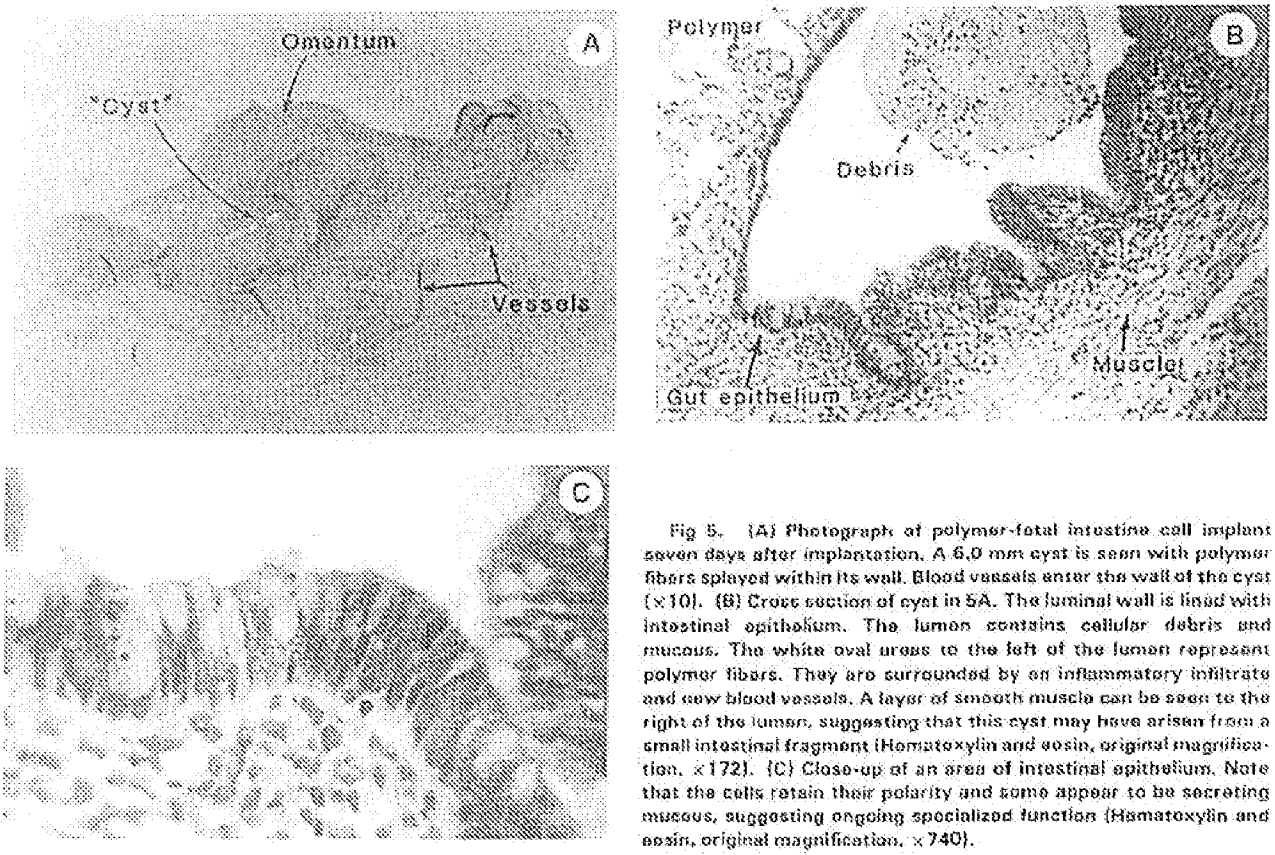

Fig 5. (A) Photograph of polymer-fetal intestine cell implant seven days after implantation. A 6.0 mm cyst is seen with polymer fibers splayed within its wall. Blood vessels enter the wall of the cyst (×10). (B) Cross section of cyst in 5A. The luminal wall is lined with intestinal epithelium. The lumen contains cellular debris and mucous. The white oval areas to the left of the lumen represent polymer fibers. They are surrounded by an inflammatory infiltrate and new blood vessels. A layer of smooth muscle can be seen to the right of the lumen, suggesting that this cyst may have arisen from a small intestinal fragment (Hematoxylin and eosin, original magnification, ×172). (C) Close-up of an area of intestinal epithelium. Note that the cells retain their polarity and some appear to be secreting mucous, suggesting ongoing specialized function (Hematoxylin and eosin, original magnification, ×740).

ing elicited an angiogenic and inflammatory response similar to their counterparts that had been seeded with parenchymal cells and maintained in culture. This suggested that the cells themselves did not play a major role in the inflammation and neovascularization seen.

DISCUSSION

Many diseases of the liver, intestine, and pancreas result in organ failure. This century has seen the development of specific pharmacologic therapy to replace lost function. Insulin replacement for diabetes mellitus is an example.

The emergence of organ transplantation and the science of immunobiology has allowed replacement of the kidney, heart, liver, and other organs. However, as our ability to perform these complex operations has improved, the limitations of the technology have become more evident. For example, in pediatric liver transplantation, donor scarcity has increased as more programs have opened. Only a small number of donors are available in the United States for 800 to 1,000 children in liver failure and those children that undergo transplantation are often so ill by the time a liver is found that the likelihood of success is diminished. The surgery is complex and usually associated with major blood loss. The preservation time is short and, therefore, results in major logistical problems in matching a distant donor with a recipient. For these reasons, the undertaking is expensive and labor intensive, requiring a major investment of resources available only in tertiary care facilities.

Selective cell transplantation of only those parenchymal elements necessary to replace lost function has been proposed as an alternative to whole or partial organ transplantation.[1] It has several attractive features. It avoids major surgery with its attendant blood loss, anesthetic difficulties and complications. It replaces only those cells that supply the needed function and, therefore, problems with passenger leukocytes, antigen presenting cells, and other cell types which may promote the rejection process are avoided. Adding the techniques of cell culture provides another set of tools to aid in the transplantation process. The ability to expand cell numbers with proliferation of cells in culture allows autotransplantation of one's own tissue. Skin equivalents using contracted collagen lattices and epidermal cells have been described.[9,10] Insertion of gene segments, and deletion of antigenic components while the cells are in culture is also possible with current technology.

Islet cell transplantation as an experimental treat-

ment of diabetes mellitus is an area of current research. Although there is evidence of short-term function, long-term results have been less satisfactory.[11,12] Currently, whole organ pancreatic transplantation is the preferred replacement. Hepatocyte injections into the portal circulation have been attempted to support hepatic function. A recent novel approach in which hepatocytes were attached to collagen-coated microcarrier beads prior to injection into the peritoneal cavity demonstrated successful implantation, viability of the implanted hepatocytes, and function. The authors suggested that cell attachment to a matrix prior to implantation was an important component of successful engraftment and function.[13]

Our studies of isolating parenchymal cells, attaching them to biodegradable polymer scaffolds in cell culture, and implanting them into hosts are based on several biologic observations:

(1) Every structure in living organisms is in a dynamic state of equilibrium. It undergoes constant renewal, remodeling, and replacement of functional tissue. The degree of change varies from organ to organ and structure to structure.

(2) Structural cells, if placed in a dissociated state, tend toward reforming structure. Their ability to do so depends on the environment in which they are placed and the degree of alteration they have undergone. Examples include capillary endothelial cells that form tubes in vitro under certain conditions[14] and bile duct cells that form tubes under the proper conditions.[15]

(3) Tissue cannot be implanted in volumes > 1.0 to 3.0 μL because nutrition is limited by the maximum diffusion distance until angiogenesis occurs.[16]

(4) Cell shape determined by cytoskeletal components and attachment to matrix plays an important role in cell division and differentiated function.[17,18] If dissociated cells are placed into mature tissue as a suspension without cell attachment, they may have a difficult time finding attachment sites, achieving polarity, and functioning because they begin with intrinsic organization. This may limit the total number of implanted cells that can remain viable to organize, proliferate, and function.

We reasoned that if we provided an organized scaffolding to which the cells were already attached, we could increase the total number of implanted cells. Artificial biodegradable polymers were chosen for several reasons. We could engineer configuration, manageability, tensile strength, and rate of degradation to a great degree with a man-made plastic and also might be able to modify the inflammatory response by modifying the material. The polymer material could be coated with other cell types or with attachment factors to increase cell attachment. In addition, we have considerable experience in placing biologically active molecules such as growth factors directly into the polymer, and allowing slow release of these agents in a controlled and predictable way.[19] Finally, by the use of biodegradable matrices, we provide only a temporary scaffold, which eventually is reabsorbed, leaving structural support of the mass to mesenchymal elements supplied by the host, and modified by the implanted cells. Although some of the polymers studied caused somewhat of an inflammatory response, it is likely that further purification will reduce or eliminate this problem. This has already been shown to be the case with ethylene-vinyl acetate[19] and polyanhydrides.[5]

This technology allowed us to design the polymers to meet the biologic needs of the system we wished to create. The configuration of the polymer scaffold must have enough surface area for the cells to be nourished by diffusion while neovascularization occurs. The new blood vessels must interdigitate with the implanted parenchymal elements to continue to support their growth, organization, and function. Polymer discs seeded with a monolayer of cells and branching fiber networks both satisfy these needs. The branching fibers are based on the same principles that nature has used to solve the problem of increasing surface area proportionate to volume increases. All multicellular organisms utilize this repeating branching structure. Branching systems represent communication networks between organs as well as the functional units of individual organs.[20] Seeding this configuration with cells and implanting the structure as fibers allows us to implant large numbers of cells, each of which is exposed to the environment of the host. Therefore, free exchange of nutrients and waste can occur while neovascularization is achieved. If functional, the new three-dimensional mass would be a true chimera of parenchymal elements of the donor, and mesenchymal elements of the recipient. The term "chimeric neomorphogenesis" describes the process that has occurred.

The results of this study demonstrate that cells from liver, intestine, and pancreas can be successfully harvested, and will attach to artificial biodegradable polymers. They will survive in culture in this configuration and can then be implanted into a host in a variety of locations. An inflammatory response that is mediated by both the wound and the nature of the polymer will occur. Successful engraftment of small clusters of hepatocytes and intestinal cells has been demonstrated. However, we do not yet have evidence of cell function in the new environment. Further studies to define the optimal characteristics of the polymer, attachment parameters, growth criteria, and function, need to be performed. Clinical application in diabetes with beta cells of the pancreas, in hepatic failure with

hepatocytes and biliary cells, and in intestinal insufficiency with intestinal epithelium remains the long-term goal.

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Discussion

P. Donahoe (Boston): This interesting approach, which uses biodegradable materials as an artificial matrix, has been successfully done in two-dimensional systems by Drs John Burke and Howard Green for the replacement of skin for major burns. Dr Vacanti is attempting this technique in a three-dimensional system. I look forward to further screening of matrix materials in the future. After maintaining dispersed cells from liver, pancreas, and intestine in culture for three days before implantation, small viable implants were found in three of 66 hepatic implants, three of 26 small intestinal implants with a 6 mm cystic structure, and 0 of 23 pancreatic implants reflecting also our own nonsuccess with that particular organ. Though falling far short of providing needed organs for transplantation, which is the author's dream, it does provide us with some promising techniques. I would make a number of suggestions for improving the rate of graft survival. First of all, allogeneic grafts were used and rejection can expect to be high. I would suggest using syngeneic mice to test the hypothesis more strongly. Ontogeny studies should be done in order to determine at which fetal age growth would be allowed. The position of implantation is also vital. We too lost a high percentage of grafts in omentum and in mesentery, as well as the hepatic capsule and in the retroperitoneum, so we turned to the subrenal capsule where structures can grow without being ejected. Can the authors tell us why the polyorthoester was so unsuccessful? Were implantation toxicity studies done before undertaking the present studies? What will be your next experimental designs? We will follow subsequent studies with great interest.

J.P. Vacanti (closing): Syngeneic mice eliminate rejection as a potential mediator of the inflammatory response, which has been significant in some of the polymers we have tested. The time-line studies with different aged animals are very important and I appreciate that point. Our hope is that adult cells can be used with success as can fetal cells. We have evolved the idea that terminal differentiation of cells is probably not very common and that in the appropriate circumstance with the appropriate communication with other cells and matrix, cells can be manipulated and can express genes that they do not usually express. We have come to the conclusion that the matrix component of different tissues probably contributes to the successful engraftment of different cell types. So

which particular location for which particular cell type needs to be worked out. We wonder whether we can take different polymers for which we have a long experience as slow release reservoirs for bioactive molecules and then use them to precondition different tissues with various growth factors and differentiation factors before we do the implant. We might be able to fool the tissue into thinking it is fetal tissue as opposed to adult tissue. The polyanhydrides at the configuration that we used were the least successful and the most toxic and they are very acidic, and it is very difficult to keep up with the acid release in culture and probably in in vivo. More work needs to be done with this particular polymer family. The polyorthoester is reasonably noninflammatory and provided good cell attachment.